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AINTEGUMENTA Promotes Petal Identity and Acts as a Negative Regulator of *AGAMOUS*

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The Arabidopsis *AINTEGUMENTA* (*ANT*) gene has been shown previously to be involved in ovule development and in the initiation and growth of floral organs. Here, we show that *ANT* acts in additional processes during flower development, including repression of *AGAMOUS* (*AG*) in second whorl cells, promotion of petal epidermal cell identity, and gynoecium development. Analyses of *ap2-1 ant-6* double mutants reveal that *ANT* acts redundantly with *AP2* to repress *AG* in second whorl cells. The abaxial surface of *ant* petals contains features such as stomata and elongated, interdigitated cells that are not present on wild-type petals. The loss of petal identity in these second whorl cells does not result from ectopic *AG* expression, suggesting that *ANT* acts in a pathway promoting petal cell identity that is independent of its role in repression of *AG.* These data suggest that *ANT* may function as a class A gene.

INTRODUCTION

Organ identity in flowers is specified by homeotic genes with activities in different regions of a developing flower. Three classes of floral homeotic genes are proposed to function in overlapping domains to specify the identity of sepals in whorl one, petals in whorl two, stamens in whorl three, and carpels in whorl four (Bowman et al., 1991; Coen and Meyerowitz, 1991; Meyerowitz et al., 1991). In Arabidopsis, the A class genes *APETALA1* (*AP1*) and *APETALA2* (*AP2*) act to specify sepal and petal development, the B class genes *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) act to specify petal and stamen development, and the class C gene *AGAMOUS* (*AG*) acts to specify stamen and carpel development. In many cases, these gene activities are restricted to particular regions by means of transcriptional regulation. Several genes controlling the establishment or maintenance of such region-specific expression have been described. The floral meristem identity gene *LEAFY* (*LFY*) is expressed throughout young floral meristems and appears to act with different region-specific factors to activate the expression of *AP3* and *AG* in particular floral whorls (Parcy et al., 1998; Busch et al., 1999).

The spatial restriction of *AG* to third and fourth whorls is controlled by several partially redundant factors, including *AP2*, *LEUNIG* (*LUG*), *CURLY LEAF* (*CLF*), and *STERILE APETALA* (*SAP*). Mutations in these genes result in various amounts of *AG* misexpression in different regions of the plant. The major repressor of *AG* in first and second whorl cells is *AP2*. Loss of *AP2* function results in ectopic *AG* expression in the outer two whorls and the corresponding homeotic transformations of these organs to carpels and stamens, respectively (Drews et al., 1991). *lug* mutants show similar but weaker homeotic transformation in the outer two whorls and have been isolated as enhancers of the weak *ap2-1* allele (Liu and Meyerowitz, 1995). In addition to the spatial expansion of the *AG* expression domain in both *ap2* and *lug* mutants, *AG* expression is initiated slightly earlier in both of these mutants. A third gene that acts to repress *AG* in whorls one and two is *SAP*. Early *sap* flowers produce sepals and small petals, whereas later flowers produce carpelloid sepals and lack second whorl organs (Byzova et al., 1999). *AG* misexpression is observed in all floral whorls and in the inflorescence meristem of *sap* mutants. *CLF* acts primarily to negatively regulate *AG* expression in vegetative tissues and to maintain repression of *AG* in flowers at older developmental stages (Goodrich et al., 1997). The predominant phenotype of *clf* mutants is the curling of leaf margins toward the midrib because of ectopic *AG* expression in leaves.

Another gene that has been speculated to play a cadastral role in restricting *AG* expression to third and fourth whorl cells is *ANT* (Elliott et al., 1996). Mutations in *ANT* cause a decrease in floral organ number and alterations in the appearance of all floral organs (Figures 1A and 1B) (Elliott et al., 1996; Klucher et al., 1996; Baker et al., 1997; Sanders et al., 1999). In addition, *ant* ovules are blocked in integument initiation and megasporogenesis (Elliott et al., 1996; Klucher et al., 1996; Baker et al., 1997; Schneitz et al., 1997). Although *ant* mutants show no homeotic transformation of organ identity, *ap2-2 ant-9* double mutants show a severe

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phenotype that resembles *ap2-9 lug-1* double mutants (Elliott et al., 1996). Flowers of these double mutants consist of a central carpel (often unfused) and an outer filament or thin bractlike structure. Both *ap2* and *ant* single mutants contain fewer floral organs than wild type. Whereas *ANT* is thought to function as a growth-promoting gene, the decreased number of floral organs in *ap2* mutants results from the growth suppression effects of ectopic *AG* expression in first and second whorl cells (Bowman et al., 1991). Two possible interpretations of the *ap2-2 ant-9* double mutant phenotype have been suggested (Elliott et al., 1996). *AP2* and *ANT* may have partially redundant functions in promoting organ initiation, or *AP2* and *ANT* may have partially redundant functions in repression of *AG*. In the latter case, the severe reduction in organ number would result from increased *AG* misexpression and its associated growth suppression. Possible redundancy between *ANT* and *AP2* is supported at the molecular level because *ANT* encodes a member of the AP2 family of transcription factors (Elliott et al., 1996; Klucher et al., 1996).

To further investigate whether *ANT* acts redundantly with *AP2* to repress *AG*, we have made double mutants with the weak *ap2-1* allele as well as other genes that act as repressors of *AG* expression. Enhancement of the *ap2-1* phenotype by *ant-6* as well as increased misexpression of *AG* in *ap2-1 ant-6* second whorl organs indicate that *ANT* does act as a redundant repressor of *AG*. In addition, *ANT* appears to be a positive regulator of specification of petal cell type because *ant-6* petals exhibit a slight loss in petal identity that is not attributable to ectopic *AG*. The roles of *ANT* in repression of *AG* and promotion of petal identity reveal that *ANT* may function as a second whorl class A gene.

RESULTS

ant Second Whorl Organs Show Partial Loss of Petal Identity

Epidermal cells on sepals, petals, stamens, and carpels show characteristic surface morphologies. Cells on the abaxial surface of sepals are of different sizes, with some quite elongated cells, some smaller cells, and stomata (Figure 2A). Cells on both the adaxial and abaxial surfaces of wild-type petals are round in appearance. Those on the adaxial surface are more cone-shaped, with cuticular thickenings running down the sides of the cone (Figure 2B), whereas those on the abaxial surface are flatter and more cobblestone-like (Figure 2C). Stomata are not present on either surface of petals. Anther epidermal cells are more irregular in shape than those of petals and are interdigitated (Figures 2D and 2E). Stomata are present on the abaxial anther surface (Figure 2E). Cells on the adaxial surface of *ant-6* petals have a morphology similar to those of wild-type petals (Figure 2F). The abaxial surface of *ant-6* petals, however, contains stomata and irregularly shaped cells (Figures 2G and 2H). These cells somewhat resemble epidermal cells of anthers as well as cells found on the abaxial surface of *clf-2* second whorl organs. The longer epidermal cells and stomata of *ant-6* petals tend to be present in the middle of the organ, whereas cells on the margins tend to resemble those found on wild-type petals. This petal phenotype is found on *ant* alleles of various strengths (*ant-4*, *ant-6*, and *ant-8*). The altered morphology of these cells reflects a loss of petal identity and perhaps a change to anther identity.

AG Expression Is Not Detected in *ant* Petals

To determine whether ectopic *AG* expression might be responsible for the *ant* second whorl abaxial epidermal cell phenotype, in situ hybridization was performed on wild-type (Landsberg *erecta* [L*er*]) and *ant* inflorescence tissue using an *AG* antisense probe. Similar patterns of *AG* expression were found in L*er*, *ant-4*, and *ant-6* flowers, the *AG* mRNA being confined to third and fourth whorl organs (Figures 3B and 3C). No *AG* expression was observed in the first or second whorls of *ant* flowers (Figure 3C). In addition, the temporal pattern of *AG* expression was unchanged in *ant* mutants. To confirm that the in situ experiments were able to detect low amounts of *AG* expression, *clf-2* tissue was hybridized alongside *ant* tissue in these experiments. Patches of *AG* expression were detected in the second whorl petals of *clf-2* mutants (Figure 3D).

The possible involvement of *AG* in the *ant* petal phenotype was also investigated by making *ag-3 ant-6* double mutants. *ag-3* plants produce indeterminate flowers consisting of the repeating pattern sepals, petals, petals (Bowman et al., 1991). The double mutant flowers consist of repeating whorls of sepals, petals, petals, but the petals resemble those found in *ant-6* with regard to organ size (Figure 1C) and cell morphology (Figures 4A to 4C). Stomata and irregularly shaped cells were detected on the abaxial surface of *ag-3 ant-6* petals (Figures 4B and 4C). The appearance of abaxial epidermal cells of *ag-3 ant-6* varied somewhat; in some cases, the cells looked rather normal, but stomata were still present (Figure 4C). The in situ experiments and the phenotype of *ag-3 ant-6* flowers indicate that the *ant* petal phenotype is not due to *AG*.

ant-6 Enhances the Second Whorl Phenotype of *ap2-1* Mutants

The weak *ap2-1* allele produces flowers with leaflike organs in whorl one, slightly staminoid petals in whorl two, stamens in whorl three, and carpels in whorl four (Figure 1D; Bowman et al., 1989). *ap2-1 ant-6* flowers produce leaflike organs in whorl one, staminoid organs in whorl two, stamens in whorl three, and carpels in whorl four (Figure 1E). Thus, *ap2-1* is epistatic to *ant-6* with regard to organ

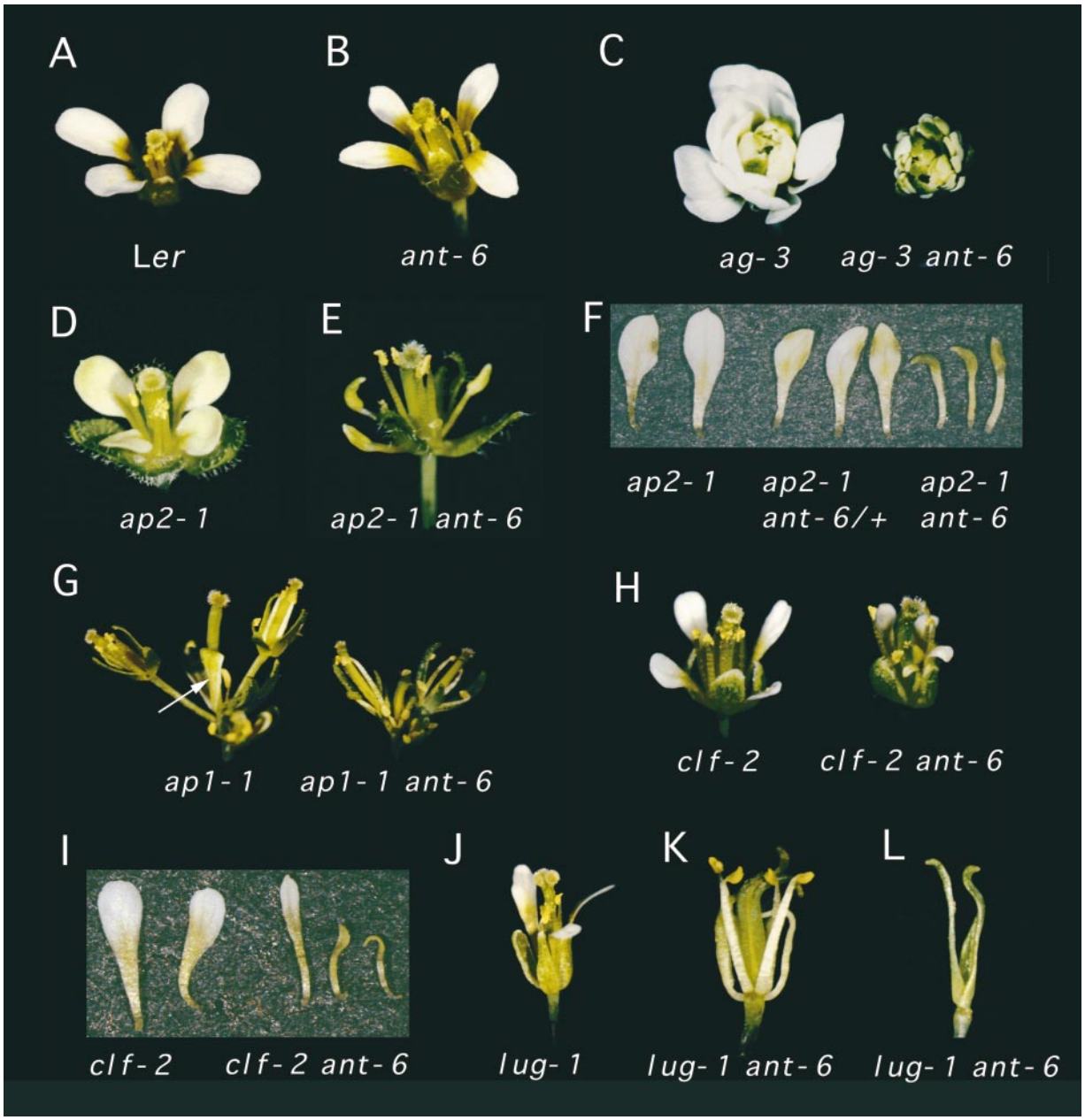

Figure 1. L*er*, *ant-6*, and *ant-6* Double Mutants.

(A) L*er*.
(B) *ant-6*.
(C) *ag-3* and *ag-3 ant-6.*
(D) *ap2-1*.
(E) *ap2-1 ant-6*.
(F) Second whorl organs from *ap2-1*, *ap2-1 ant-6*/+, and *ap2-1 ant-6*.
(G) *ap1-1* and *ap1-1 ant-6*. Arrow indicates a petaloid organ in *ap1-1*.
(H) *clf-2* and *clf-2 ant-6*.
(I) Second whorl organs from *clf-2* and *clf-2 ant-6*.
(J) *lug-1*.
(K) *lug-1 ant-6*.
(L) *lug-1 ant-6* fourth whorl.

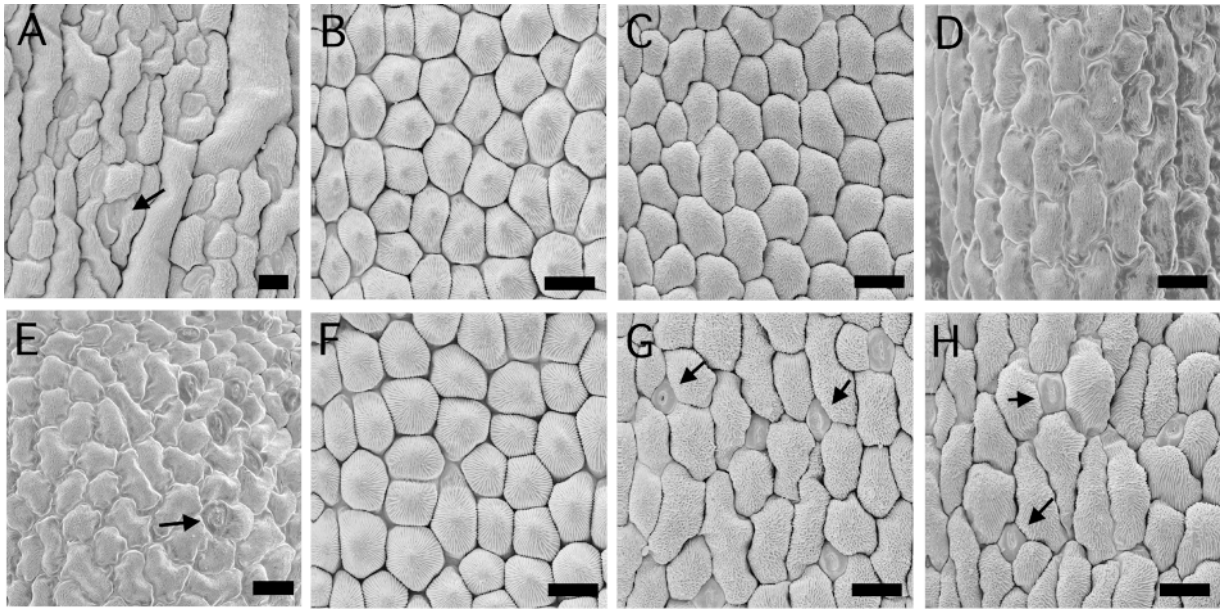

Figure 2. Scanning Electron Micrographs of L*er* and *ant-6* Floral Organs.

(A), **(C)**, and **(E)** Abaxial epidermis of sepal **(A)**, petal **(C)**, and anther **(E)** from L*er*.
(B) and **(D)** Adaxial epidermis of petal **(B)** and anther **(D)** from L*er*.
(F) Adaxial epidermis of an *ant-6* petal.
(G) and **(H)** Abaxial epidermis of an *ant-6* petal.
Arrows indicate stomata. Bars = 10 μm.

identity in whorl one. Organs in whorls one, three, and four resemble *ant-6* organs in their morphology; they tend to be narrow, and the stamens consist of two locules rather than four. In addition, organ number is decreased in the double mutant. An average of 3.5, 2.9, 4.1, and 2.0 floral organs are found in whorls one to four of the double mutant, compared with an average of 4.0, 3.3, 5.4, and 2.0 organs in the respective whorls of *ap2-1*.

Second whorl organs in the double mutant show an enhanced transformation to staminoid organs (Figure 1F). The earliest-formed second whorl organs were leaflike, petallike, or stamenlike in overall appearance (Figures 5B, 5J, and 5K) with cell types characteristic of all of these organs present on the adaxial surface (Figure 5F). The abaxial surface tended to be more leaflike, with some cells lacking epicuticular thickenings (Figure 5O), and trichomes occasionally were present. Often, the abaxial surface of these organs had cell types similar to those found on the abaxial surface of *ap2-1* organs (Figures 5M and 5N). In later-arising *ap2-1 ant-6* flowers, the second whorl organs were more staminoid in overall appearance (Figures 5C, 5D, and 5L). These second whorl organs often possessed loculelike structures (Figure 5D) with cell types characteristic of anthers (Figure 5H). The abaxial surface of these organs often remained leaflike but sometimes had cells that were more staminoid in shape than those on *ap2-1* organs (Figure 5P). The transformation to stamen identity was not total: only some of these second whorl organs produced pollen.

Flowers of plants with the genotype *ap2-1 ant-6*/+ showed a slightly more severe phenotype than *ap2-1* flowers. The second whorl organs tended to be somewhat smaller, more pointed, and more yellow in color (Figure 1F). Thus, *ant-6* becomes partially dominant in an *ap2-1* background. No phenotypic differences were observed between *ap2-1*/+ ant-6 flowers and those of *ant-6*.

Enhancement of *ap2-1* Phenotype Correlates with Increased *AG* Expression

The presence of *AG* RNA in first and second whorl organs of *ap2-1* flowers has been described previously (Drews et al., 1991). In those experiments, low amounts of *AG* RNA were detected in *ap2-1* first whorl cells, and patches of *AG* RNA were detected in some *ap2-1* second whorl organs. To determine whether the increased stamen identity of *ap2-1 ant-6* second whorl organs is the result of increased amounts of *AG* ectopic expression in these organs, the pattern of *AG* expression was examined in *ap2-1* and *ap2-1 ant-6* flowers. *AG* expression was first observed in stage 3 flowers of *ap2-1*

(Figure 3E) and *ap2-1 ant-6* (Figure 3I) in cells of the floral meristem but not in the young first whorl primordia, similar to the pattern observed in wild-type flowers (Figure 3A). At later stages in flower development, *AG* RNA was detected in patches of adaxial epidermal cells (Figures 3F and 3H) and occasionally in some subepidermal layers on the adaxial side of second whorl organs. The pattern of *AG* expression in *ap2-1 ant-6* second whorl organs was somewhat expanded. Patches of *AG* RNA were detected more frequently in subepidermal tissue of the double mutant (Figures 3J to 3L). Expression was sometimes seen on the abaxial side of *ap2-1 ant-6* second whorl organs. In addition, the frequency of *AG* expression in *ap2-1 ant-6* second whorl organs was greater than in *ap2-1*. In flowers of stages 6 through 10, *AG* expression was observed in 18 of 19 second whorl organs from *ap2-1 ant-6* and in 9 of 17 second whorl organs from *ap2-1*. The enhanced staminoid appearance of the adaxial surface of *ap2-1 ant-6* second whorl organs in comparison with the abaxial surface is consistent with the increased presence of *AG* ectopic expression in these regions of second whorl organs.

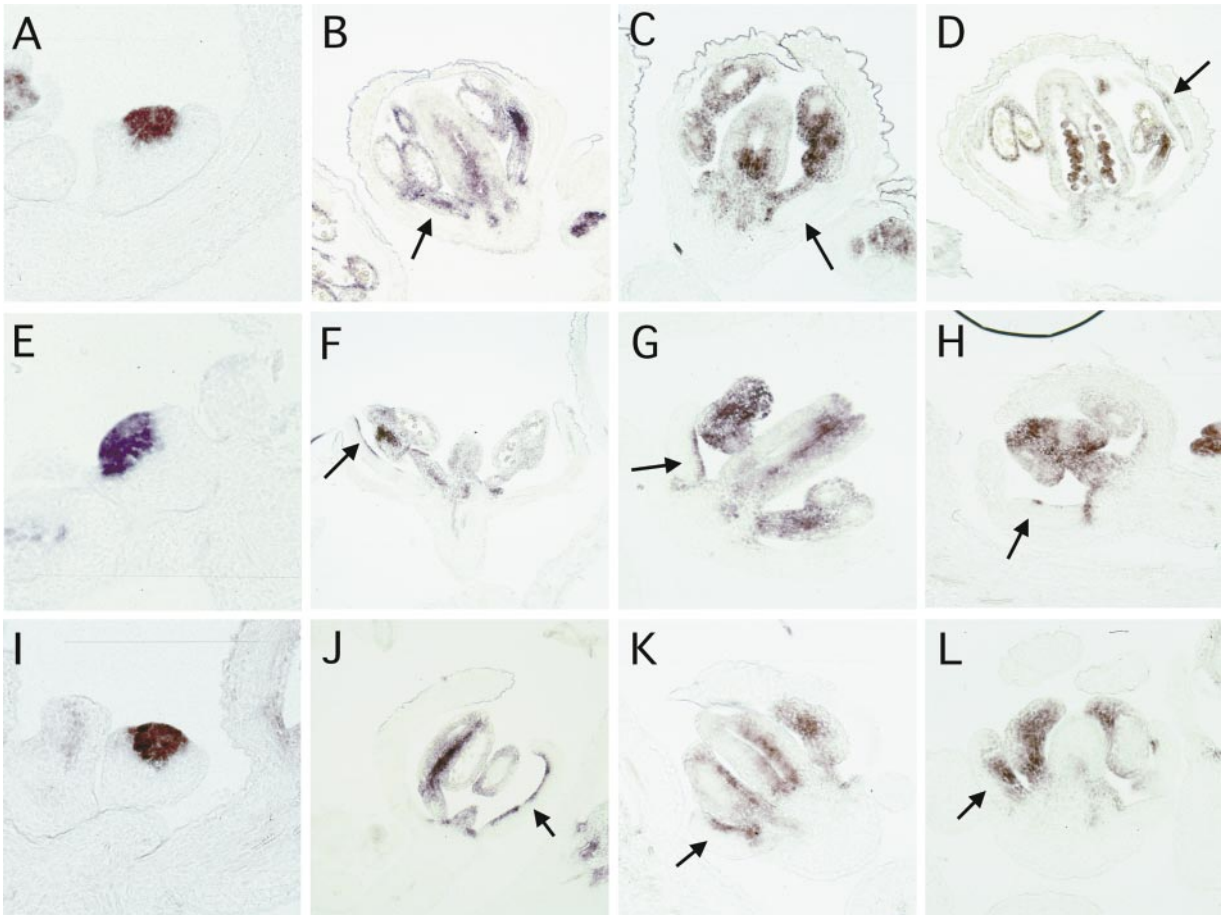

Figure 3. Localization of *AG* RNA in Longitudinal Sections of Flowers from L*er*, *ant-4*, *clf-2*, *ap2-1*, and *ap2-1 ant-6* by in Situ Hybridization.

Arrows indicate second whorl organs that either show no *AG* expression (**[B]** and **[C]**) or that do exhibit ectopic *AG* expression (**[D]**, **[F]**, **[G]**, **[H]**, **[J]**, **[K]**, **[L]**).

(A) Stage 3 wild-type (L*er*) flower.

(B) Stage 10 wild-type (L*er*) flower.

(C) Stage 9 *ant-4* flower.

(D) Stage 12 *clf-2* flower.

(E) Stage 3 *ap2-1* flower.

(F) to **(H)** *ap2-1* flowers at stages 8 to 13. *AG* RNA is detected in patches of epidermal cells of second whorl organs.

(I) Stage 3 *ap2-1 ant-6* flower.

(J) to **(L)** *ap2-1 ant-6* flowers at stages 8 to 12. *AG* RNA is detected in epidermal and some subepidermal cells of *ap2-1 ant-6* second whorl organs.

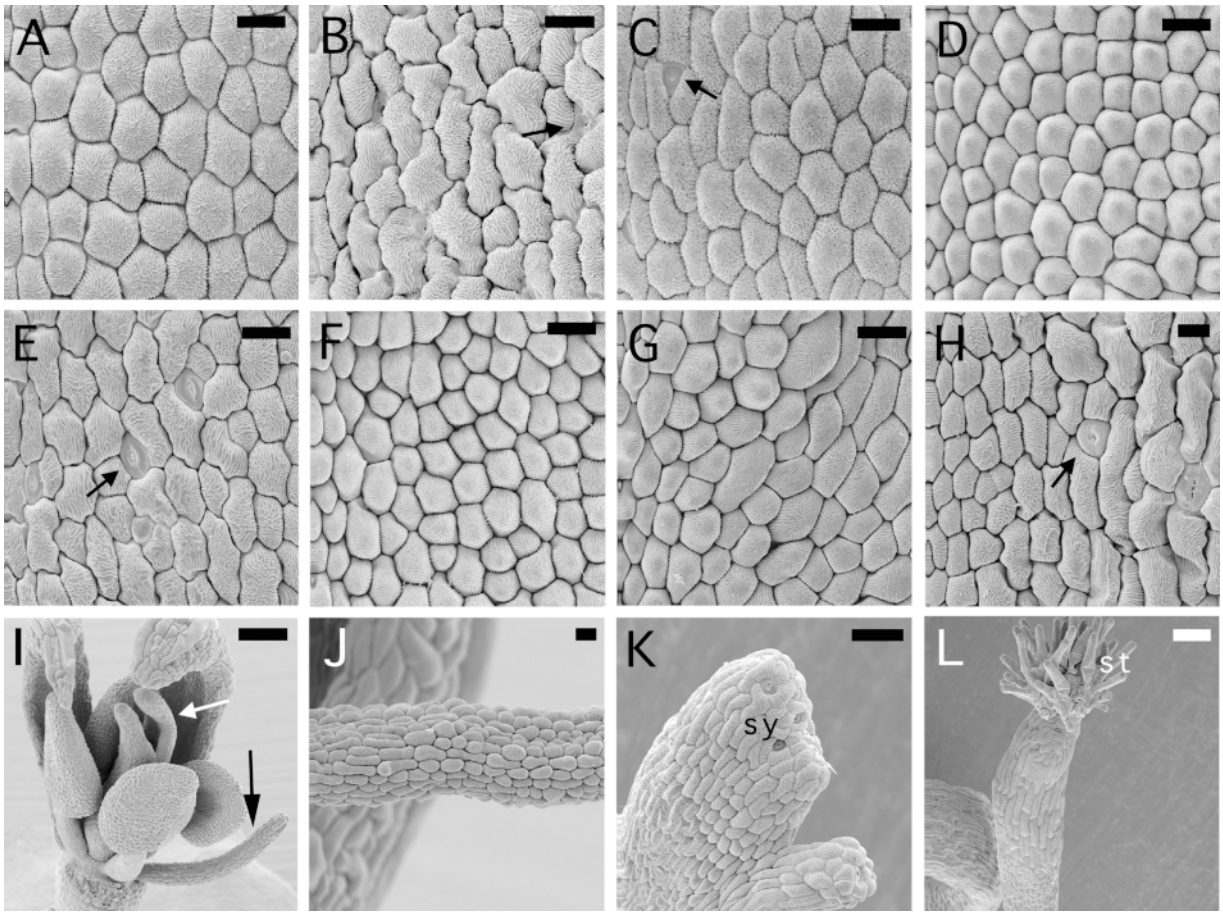

Figure 4. Scanning Electron Micrographs of *ag-3*, *ag-3 ant-6*, *clf-2*, *clf-2 ant-6*, and *lug-1 ant-6* Flowers.

(A) Abaxial epidermis of an *ag-3* petal.
(B) and **(C)** Abaxial epidermis of an *ag-3 ant-6* petal. Stomata and elongated, interdigitated cells are present on these second whorl organs.
(D) and **(E)** Adaxial **(D)** and abaxial **(E)** epidermis of a *clf-2* petal.
(F) Adaxial epidermis of a *clf-2 ant-6* second whorl organ.
(G) Adaxial epidermis of a *clf-2 ant-6* second whorl organ with some cone-shaped cells as well as some flattened cells.
(H) Abaxial epidermis of a *clf-2 ant-6* petal.
(I) *lug-1 ant-6* flower with a filament in the second whorl (black arrow) and an unfused gynoecium (white arrow).
(J) Petaloid filament found in the second whorl of a *lug-1 ant-6* flower.
(K) Apical region of a *lug-1 ant-6* gynoecia with horn-like protrusions consisting of stylelike tissue (sy).
(L) Hornlike protrusion of a *lug-1 ant-6* gynoecium topped with stigmatic papillae (st).
Arrows in **(B)**, **(C)**, **(E)**, and **(H)** indicate stomata. Bars in **(A)** to **(H)** and **(J)** = 10 μm; bars in **(I)** and **(L)** = 50 μm; bar in **(K)** = 30 μm.

Decreased Petal Identity in *ap1-1 ant-6* and *clf-2 ant-6* Double Mutants

ap1-1 flowers contain leaflike organs in whorl one, stamens in whorl three, and carpels in whorl four and are usually missing second whorl organs. In addition to these defects in floral organ identity, *ap1-1* flowers produce additional flowers in the axils of the first whorl organs (Figure 1G) (Irish and Sussex, 1990; Bowman et al., 1993). *ap1-1 ant-6* double mutants resemble *ap1-1* with regard to floral organ identity and resemble *ant-6* with respect to floral organ and ovule morphology (Figure 1G). Although petals and petaloid organs are rarely found in *ap1-1* primary flowers, such organs are fairly common in secondary and higher order flowers (averaging 3.8 petals or petaloid organs per flower on 33 *ap1-1* flowers counted). In contrast, the number of petaloid

organs is severely reduced in *ap1-1 ant-6* double mutants (averaging 0.33 petals or petaloid organs per flower on 36 *ap1-1 ant-6* flowers counted). The double mutants also exhibit less internode elongation between primary and secondary flowers (Figure 1G).

Mutations in *CLF* result in curled leaves, early flowering, and flowers that show slight transformations of first whorl organs to carpels and of second whorl organs to stamens (Goodrich et al., 1997). In our growth conditions, the *clf-2* phenotype was less severe, with fairly normal first whorl organs and small petals found in the second whorl (Figure 1H). Cells on the adaxial surface of *clf-2* petals were similar to those on wild-type petals (Figure 4D). However, stomata and more irregularly shaped cells were present on the abaxial surface of *clf-2* petals (Figure 4E). *ant-6 clf-2* double mutants exhibit the curled leaves and early flowering of *clf-2* mutants and produce floral organs in whorls one, three, and four that resemble those in *ant-6* mutants (Figure 1H). *clf-2 ant-6* second whorl organs vary in appearance, with some flowers exhibiting organs resembling very narrow petals and others having quite small, yellow organs (Figure 1I). The adaxial surface of *clf-2 ant-6* second whorl organs often resembles wild-type petals with conical-shaped cells (Figure 4F), but in some cases, flattened cells are also present (Figure 4G).The abaxial surface of *clf-2 ant-6* second whorl organs appears similar to that of the single mutants (Figure 4H).

Reduction of Petals and Elimination of Some Gynoecium Tissues in *lug-1 ant-6* Double Mutants

Mutations in *LUG* result in a reduction in organ number and the production of narrow floral organs that somewhat resemble those of *ant* mutants (Figure 1J; Liu and Meyerowitz, 1995). In addition, first whorl organs are sometimes staminoid or petaloid, and second whorl organs are often absent. Later flowers show more severe organ identity transformations than do earlier flowers. Defects in carpel fusion are also present in *lug-1*, with hornlike protrusions often present at the top of the carpel valves (Liu and Meyerowitz, 1995). Narrow sepals and stamens with two locules are found in the first and third whorls, respectively, of *lug-1 ant-6* flowers (Figure 1K). *lug-1 ant-6* flowers show a synergistic phenotype in the second and fourth whorls. Second whorl organs are usually missing, although very thin petals, petaloid filaments, or filaments are sometimes produced (Figures 1K, 4I, and 4J). The fourth whorl of *lug-1 ant-6* plants consists of two carpels that are partially or completely unfused on one or both sides (Figure 1L). *lug-1 ant-6* gynoecia show a marked decrease in stigmatic tissue and are completely missing all adaxial tissues, including ovules, placenta, and septa. The tops of *lug-1 ant-6* gynoecia consist of pointed projections with epidermal cells characteristic of styles (Figure 4K). In a few cases, stigmatic papillar cells are present at the tip of these projections (Figure 4L).

DISCUSSION

ANT Is a Redundant Repressor of *AG* in Second Whorl Cells

Previous characterization of the phenotype of *ap2-2 ant-9* double mutants had suggested that *ANT* might play a role in *AG* repression (Elliott et al., 1996). This is now further supported by the phenotype of *ap2-1 ant-6* flowers. Several differences exist between *ANT* and other cadastral regulators of *AG* expression. Unlike mutations in *AP2, LUG, CLF,* and *SAP*, the *ant* single mutants show no obvious homeotic transformation of organ identity, indicating that the role of *ANT* in *AG* repression is entirely redundant with that of *AP2*. In addition, *ANT* appears to act as a second whorl–specific repressor of *AG*. Unlike *AP2* and *LUG*, *ANT* is not involved in controlling the initiation of *AG* expression. *AG* expression is initiated in a normal pattern in stage 3 floral meristems of *ap2-1 ant-6* plants.

The enhancement of the *ap2-1* phenotype when *ant-6* is heterozygous suggests that the amount of ANT activity is critical in flowers compromised for AP2 activity and might indicate that ANT and AP2 interact directly or at least work in the same pathway. Previously, dominant interactions have been observed between *lug* and *ap2* and between *clf* and *ap2* (Liu and Meyerowitz, 1995; Goodrich et al., 1997). So far, all of the identified *AG* repressors have homology to either transcription factors (AP2, ANT, and SAP) (Elliott et al., 1996; Klucher et al., 1996; Byzova et al., 1999) or to a chromatin-associated protein (CLF) (Goodrich et al., 1997). One possibility is that some or all of these proteins form a complex involved in transcriptional repression of *AG*. Because of its severe mutant phenotype, AP2 is expected to be a critical subunit of such a complex. This complex might associate with chromatin-associated proteins to keep *AG* in a transcriptionally silent state in particular cells. Recently, *AP2* has been shown to negatively regulate *AG* expression through several independent enhancer elements (Bomblies et al., 1999). The fact that multiple regulatory elements require AP2 activity for preventing *AG* expression in floral whorls one and two supports the idea that these factors might not work independently through different cis-acting elements but rather as a complex.

ANT Acts to Promote Petal Cell Identity Independently of Its Role in *AG* Repression

Although *ant* single mutants do not show homeotic alterations in organ identity, some second whorl abaxial epidermal cells lose petal identity. The presence of stomata and the morphology of the elongated cells suggest a transformation to anther identity, even though *AG* expression was not detected here. This suggests that *ANT* acts to repress other class C genes involved in specifying stamen identity or that

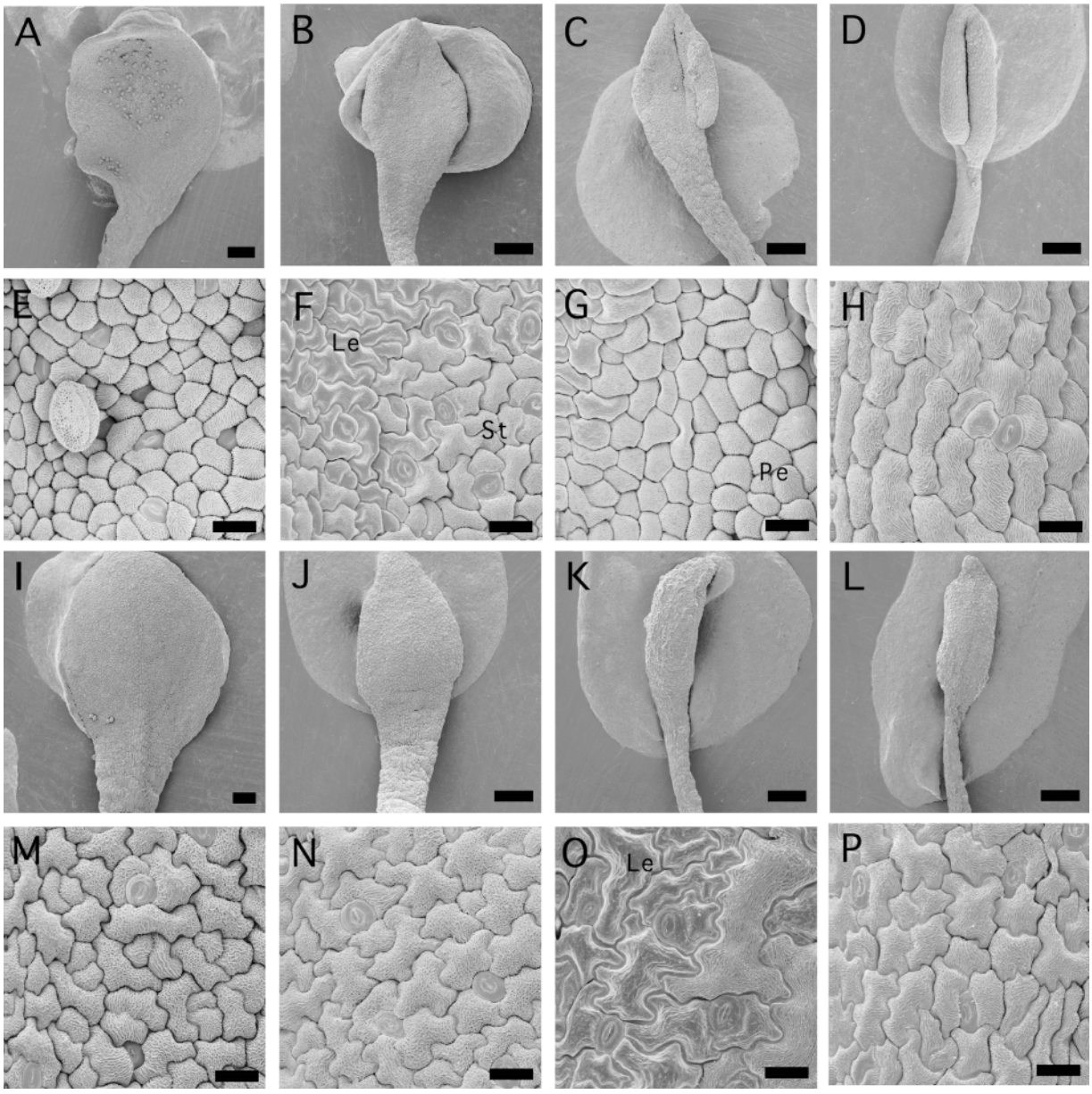

Figure 5. Scanning Electron Micrographs of *ap2-1* and *ap2-1 ant-6* Flowers.

(A) Low magnification of the adaxial surface of an *ap2-1* second whorl organ.
(B) to **(D)** Low magnification of the adaxial surfaces of *ap2-1 ant-6* second whorl organs, showing various degrees of stamenlike features.
(E) High magnification of the adaxial surface of the *ap2-1* second whorl organ shown in **(A)**.
(F) to **(H)** High magnification of the adaxial surface of *ap2-1 ant-6* second whorl organs shown in **(B)** to **(D)**, respectively, with leaflike, petal-like, and stamenlike cells indicated.
(I) Low magnification of the abaxial surface of an *ap2-1* second whorl organ.
(J) to **(L)** Low magnification of the abaxial surfaces of *ap2-1 ant-6* second whorl organs.
(M) High magnification of the abaxial surface of the *ap2-1* second whorl organ shown in **(I)**.
(N) to **(P)** High magnification of the abaxial surfaces of *ap2-1 ant-6* second whorl organs shown in **(J)** to **(L)**, respectively.
Le, leaflike cells; Pe, petal-like cells; St, stamenlike cells. Bars in **(A)** to **(D)** and **(I)** to **(L)** = 100 μm; bars in **(E)** to **(H)** and **(M)** to **(P)** = 10 μm.

ANT acts to promote petal identity in these cells. If *ANT* does act to repress other class C genes, these genes must function independently of *AG* because *ag-3 ant-6* petals exhibit a phenotype similar to that of *ant-6* petals.

That *ANT* might function as a class A gene is supported by its role in *AG* repression and by the phenotype of the *ap1-1 ant-6* double mutant, in which organs with any petal characteristics are almost completely eliminated. In this case, the *ant* second whorl abaxial phenotype could be explained by a decreased class A function with no corresponding expansion of class C function, given that AP2 activity remains. B function in the absence of A and C functions produces organs that are intermediate between petals and stamens and cells with intermediate morphologies (Bowman et al., 1991). If *ANT* does possess class A organ identity activity, this function is almost completely redundant with that of *AP1* and *AP2*, such that *ant* mutants show loss of petal identity in only one particular cell type. Unlike the other Arabidopsis A function genes, the roles of *ANT* in both organ identity specification and *AG* repression are restricted to a single whorl. Alternatively, *ANT* may not function as a class A gene but rather as a specifier of a particular petal cell type. In this role, ANT would function downstream of the class A and B gene activities. Consistent with this proposed role, *ANT* is expressed at late stages in the petal abaxial epidermis (Elliott et al., 1996).

Possible Roles of Class C Repressors in Evolution of Petals

Most of the known *AG* repressors appear to be multifunctional genes that play several roles during flower development. *LUG* and *ANT* are involved in organ growth (Liu and Meyerowitz, 1995; Elliott et al., 1996; Klucher et al., 1996), *AP2* and *SAP* have roles in specifying floral meristem identity (Bowman et al., 1993; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993; Byzova et al., 1999), and *AP2*, *LUG*, *SAP*, and *ANT* are all involved in ovule development (Modrusan et al., 1994; Elliott et al., 1996; Klucher et al., 1996; Baker et al., 1997; Roe et al., 1997; Schneitz et al., 1997; Byzova et al., 1999). Although these genes have slightly different roles with respect to the precise timing and spatial restriction of *AG* expression, the presence of so many known regulators of *AG* expression seems odd, considering that few regulators of class B gene expression have been identified. Because ovules are evolutionarily older than flowers, these genes may have initially had roles in ovule development and were recruited for other roles in flower development at later times (Theissen et al., 2000).

Petals are hypothesized to have arisen independently several times from stamens (andropetals) or sterile bracts (bracteopetals) in different angiosperm lineages (Takhtajan, 1991). The evolution of andropetals from a duplicated whorl of stamens might have involved genes that repressed C function in this region of the flower. Some of these genes later may have acquired functions in specifying petal organ identity. Because of the importance of petals in pollination, it might have been advantageous for these plants to maintain several repressors of C function. It will be interesting to see whether *AP2*, *ANT*, *LUG*, and *SAP*-like genes are present in different angiosperms thought to have andropetals versus bracteopetals and whether these genes function in repression of class C genes and petal development in these different lineages.

ANT Functions in Many Different Processes during Flower Development

Consistent with its broad expression pattern in both vegetative and reproductive organs, *ANT* appears to function in several different developmental processes (Elliott et al., 1996; Klucher et al., 1996; Baker et al., 1997). On the basis of its single-mutant phenotype, *ANT* was shown to play a role in floral organ initiation and growth as well as integument initiation in ovules. Several additional roles have been uncovered through the analysis of double mutants. For example, *ANT* acts redundantly with *HUELLENLOS* (*HLL*) to specify ovule outgrowth along the proximal–distal axis (Schneitz et al., 1998). Here, we show that *ANT* acts redundantly with *AP2* to repress *AG* in second whorl cells and in fact may function as a class A gene with regard to specification of petal identity. Isolation of downstream target genes as well as of interacting proteins should help illuminate the manner in which this transcription factor works within these different developmental pathways.

METHODS

Strain Constructions

All double mutants were created by pollinating carpels from heterozygous *ag-3* and homozygous *ap2-1*, *ap1-1*, *lug-1*, and *clf-2* with pollen from *ant-6* homozygotes. *ant-6* is a putative null allele (Krizek, 1999). All *ant-6* double mutants could be identified by the presence of anthers with two locules. Because *ANT* is closely linked to *AP2* and *LUG*, double mutants were identified in the F_3 of these crosses. Seeds from 30 individual F_1 plants from the *ap2-1* cross were planted as individual families. Of the ~500 total plants from these families, one showed a noticeably more severe phenotype than *ap2-1*. Genotyping of this plant by polymerase chain reaction (Krizek, 1999) showed it to be heterozygous for *ant-6*. Progeny from this plant segregated the *ap2-1 ant-6* double mutant.

Scanning Electron Microscopy

Samples for scanning electron microscopy were prepared and examined as previously described (Krizek, 1999).

In Situ Hybridization

Nonradioactive in situ hybridizations were performed as previously described (Krizek, 1999). *AG* antisense probe was made by linearization of pCIT565 (Yanofsky et al., 1990) with HindIII and in vitro transcription with T7 RNA polymerase. *ap2-1* and *ap2-1 ant-6* tissues of the same age were sectioned and put side by side on a slide for hybridization with the *AG* probe.

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